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SPECIALTY SECTION

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

RECEIVED 16 June 2022

ACCEPTED 25 August 2022

PUBLISHED 23 September 2022

CITATION

Salgado FF, da Silva TLC, Vieira LR,
Silva VNB, Leão AP, Costa MMDC,
Togawa RC, de Sousa CAF, Grynberg P and
Souza MT Jr (2022) The early response of
oil palm (*Elaeis guineensis* Jacq.) plants to
water deprivation: Expression analysis of
miRNAs and their putative target genes,
and similarities with the response to salinity
stress.

Front. Plant Sci. 13:970113.

doi: 10.3389/fpls.2022.970113

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The early response of oil palm (*Elaeis guineensis* Jacq.) plants to water deprivation: Expression analysis of miRNAs and their putative target genes, and similarities with the response to salinity stress

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Oil palm (*Elaeis guineensis* Jacq.) is a oilseed crop of great economic importance drastically affected by abiotic stresses. MicroRNAs (miRNAs) play crucial roles in transcription and post-transcription regulation of gene expression, being essential molecules in the response of plants to abiotic stress. To better understand the molecular mechanisms behind the response of young oil palm plants to drought stress, this study reports on the prediction and characterization of miRNAs and their putative target genes in the apical leaf of plants subjected to 14 days of water deprivation. Then, the data from this study were compared to the data from a similar study that focused on salinity stress. Both, the drought- and salt-responsive miRNAs and their putative target genes underwent correlation analysis to identify similarities and dissimilarities among them. Among the 81 identified miRNAs, 29 are specific for oil palm, including two (*egu-miR28ds* and *egu-miR29ds*) new ones – described for the first time. As for the expression profile, 62 miRNAs were significantly differentially expressed under drought stress, being five up-regulated (*miR396e*, *miR159b*, *miR529b*, *egu-miR19sds*, and *egu-miR29ds*) and 57 down-regulated. Transcription factors, such as MYBs, HOXs, and NF-Ys, were predicted as putative miRNA-target genes in oil palm under water deprivation; making them the most predominant group of such genes. Finally, the correlation analysis study revealed a group of putative target genes with similar behavior under salt and drought stresses. Those genes that are upregulated by these two abiotic stresses encode lncRNAs and proteins linked to stress tolerance, stress memory, modulation of ROS signaling, and defense

response regulation to abiotic and biotic stresses. In summary, this study provides molecular evidence for the possible involvement of miRNAs in the drought stress response in oil palm. Besides, it shows that, at the molecular level, there are many similarities in the response of young oil palm plants to these two abiotic stresses.

KEYWORDS

abiotic stress, tolerance, transcriptome, transcription factor, non-coding RNA, lncRNA

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a palm tree from the Arecaceae family, classified as one of the most productive oil seed crops (Wang, L. et al., 2020). Originally from West Africa, it has been successfully introduced and exploited commercially in Asia, Africa, and Latin America. Indonesia and Malaysia are the largest palm oil-producing nations (EPOA, 2020). This species has great economic importance due to the high fruit production and oil efficiency extraction, with refining processes that render both palm oil and palm kernel oil (Corley, 2009; Silva et al., 2016). Palm oil is the raw material for cosmetics, medicines, candles, soaps, biofuels, and lubricating greases, and its demand is increasing (Abrapalma, 2018).

Oil palm does not withstand long periods of severe or moderate drought, and its fruit yield decreases considerably under water scarcity (Azzeme et al., 2016). It requires ~2,000 mm/year of water and does not tolerate drought for more than 90 days (Corley et al., 2018). According to Silva et al. (2016, 2017), water stress from seven to 21 days induced physiological changes and affected the growth of oil palm seedlings; while repetitive water deficit events induced photosynthetic acclimation in young oil palm plants (Lopes Filho et al., 2021). However, there is not much information about the molecular mechanisms behind the responses of oil palm plants to drought stress.

MicroRNAs (miRNAs) are small molecules of non-protein-coding RNAs, 20 to 24 nucleotides (nts) in length, derived from single-stranded precursors, which form a secondary stem-loop structure (Xin et al., 2015). They are involved in gene expression regulation at a post-transcription level in plants, animals, fungi, and viruses (Denli et al., 2004; Lytle et al., 2007; Ventura et al., 2008; Xu et al., 2019). miRNAs molecules are highly conserved in plants (da Silva et al., 2016).

Several studies have shown that miRNAs are involved in many biological and metabolic processes (Comai and Zhang, 2012; Sun, 2012). They play crucial roles in plant growth regulation (Mallory et al., 2004), flower development (Chen, 2004; Zhu et al., 2009), and responses to abiotic stresses, such as drought and salinity (Liu et al., 2008; Ding et al., 2009; Lv et al., 2010; Chen et al., 2018; Qiu et al., 2020; Salgado et al., 2021; Zeeshan et al., 2021).

Water stress-responsive miRNAs are present in *Oryza sativa* (Zhou et al., 2010), *Medicago truncatula* (Wang et al., 2011), and *Arabidopsis thaliana* (Liu et al., 2008). Zhou et al. (2010) described 11 down- and eight up-regulated miRNAs in rice plants under drought stress. Li et al. (2008) reported that miR169a and miR169c are significantly down-regulated by drought, promoting increased drought resistance in arabidopsis. They postulated that it was because one of the miR169 targets, NFYA5 (Nuclear Factor YA5), is a crucial transcription factor that regulates the expression of numerous drought stress-responsive genes (Li et al., 2008).

Studies have shown that miR398 and miR408 are positively regulated by water deficit in *M. truncatula*, leading to the negative regulation of its target genes (COX5b, CSD1, and plantacyanin; Trindade et al., 2010). In *Zea mays*, miRNAs modulate the expression of MAPK (mitogen-activated protein kinase), PLD (phospholipase D), PHD (proline dehydrogenase), and POD (peroxidase; Wei et al., 2009); which are known to be involved in plant response to environmental stresses as part of signaling pathways (MAPK and PLD) or as having role in the ROS-scavenging system (PHD and POD).

Although some studies identified miRNAs in oil palm (Md Nasaruddin et al., 2007; Xiao et al., 2013; Low et al., 2014; da Silva et al., 2016; Salgado et al., 2021), only a few miRNAs from *E. guineensis* are in miRBase (version 2.1). To the best of our knowledge, there are no reports on prospecting and characterizing drought-responsive miRNAs in oil palm. This study reports on the prediction and characterization of miRNAs responsive to drought stress in oil palm plants and their target genes. Besides, the drought- (from this study) and salt-responsive (from Salgado et al., 2021) miRNAs and their putative target genes underwent correlation analysis to identify similarities and dissimilarities among them.

Materials and methods

Plant material, growth conditions, experimental design, and drought stress

The oil palm plants used in this study were clones regenerated out of embryogenic calluses (Corrêa et al., 2015) obtained from

leaves of an adult plant belonging to the *E. guineensis* genotype AM33, a Deli x Ghana from ASD Costa Rica.¹ The plants used in this study were different of the one used in the Salgado et al. (2021) study; although, all plants – from both studies – came from the same embryogenic calluses; consequently, they are all clones of the same plant.

The embryogenic calluses were transferred to a regeneration medium on January 2016 and kept in a BOD chamber at 30°C and a 16/8-h light/dark photoperiod. On July and December 2016, the plants regenerated *in vitro* were put in 200-mL plastic cups containing vermiculite and a commercial substrate (Bioplant® – Bioplant Agrícola Ltda., Nova Ponte, MG, Brazil), in a 1:1 ratio, on a dry basis; transferred to the PGW40 growth chamber (Conviro, Winnipeg, Canada), with air temperature at 25 ± 2°C, relative humidity at 60 ± 10%, and light intensity at 500 ± 50 μmol m⁻² s⁻¹, for acclimation; and then transferred to a greenhouse. Once acclimated, they were transferred to black plastic pots (3.5 L) containing 1,200 g of vermiculite, soil, and a commercial substrate (Bioplant®) mix, in a 1:1:1 ratio – on a dry basis; and fertilized using 2.5 g/L of the formula nitrogen (N), phosphorus (P), and potassium (K) fertilizer 20-20-20.

Before starting the experiments, plants were screened accordingly to their development stage, size, and number of leaves, to use the most uniform group of plants possible. The experiment was performed in a greenhouse in Brasília, DF, Brazil (S-15.732°, W-47.900°). Main environmental variables (temperature, humidity, and radiation), measured at a nearby meteorological station (S-15.789°, W-47.925°), fluctuated according to the weather conditions. Plants underwent drought stress in the growth stage known as bifid saplings.

Two experiments were carried out in November 2017 (Experiment 1) and March 2018 (Experiment 2), and both consisted of two treatments: a control one, with four replicates, and a drought stress one, with six replicates. The experimental design was completely randomized blocks. All plants in the control treatment were maintained at field capacity throughout the entire experiment, while plants in the drought stress treatment were initially at field capacity, and then they were deprived of water addition for 14 days.

Evapotranspiration rate and gas exchange measurements

Plant weight was measured daily and individually to determine the water lost by evapotranspiration. The weight of the vessels containing control plants was measured, and the soil received water to field capacity, with no water added in the remaining treatment. The daily evapotranspiration average measured before the onset of experiments 1 and 2 was considered 100%. The gathered data was then calculated from this initial

value, as follows: evapotranspiration (%) = day evapotranspiration (mL) × 100 (%) / mean evapotranspiration before stress (mL). After submitting the data for normality analyses using the Shapiro–Wilk test, an statistical analysis was performed by the Student's *t*-test at 5% probability using GraphPad.

The parameters of leaf gas exchange [net CO₂ assimilation rate (*A*), transpiration rate (*E*), stomatal conductance to water vapor (*g_s*), and intercellular CO₂ concentration (*C_i*)] were measured using a portable infrared gas analyzer LI-COR Mod. 6400XT (LI-COR, Lincoln, NE, United States) equipped with a measuring chamber (2 × 3 cm) with artificial light system LI-COR Mod. 6400-02B. We used the OPEN software version 6.3 to extract the data. The block temperature was 25°C, PAR was 1,500 μmol/m²/s, the relative humidity of the air inside the measuring chamber was between 50 and 60%, the airflow index was 400 μmol/s, and the CO₂ concentration was 400 ppm in the reference cell, using the model 6400-01 CO₂ mixer with cylinder CO₂ (7.5 g). After submitting the gas exchange data to the Kruskal–Wallis test, we applied the Dunn's test (*p* < 0.05) to those data with significant differences between treatments. The gas exchange measurements were on the middle third of the apical leaf, in a previously marked area, between 9:00 and 11:00 a.m., only in the first experiment.

Transcriptomics analysis

Apical leaves from three control and three drought-stressed plants, collected 14 days after setting up the treatments (DAT), were immediately immersed in liquid nitrogen and stored at –80°C until RNA extraction, library preparation, and sequencing. Total RNA isolation, as well as the RNA quantity and quality analysis, were performed as described in Salgado et al. (2021). The GenOne Company (Rio de Janeiro, RJ, Brazil) performed the RNA-Seq using an Illumina HiSeq platform and the paired-end strategy. The Functional Genomics Center / ESALQ-USP (Piracicaba, SP, Brazil) performed the small RNAs sequencing using an Illumina HiSeq platform.

The OmicsBox version 1.3 (OmicsBox, 2019) was employed to perform all RNA-Seq analyses, using the same pipeline of analysis described previously in Salgado et al. (2021). Here, we also used FastQC (Andrews, 2018) and Trimmomatic (Bolger et al., 2014) for quality control, STAR (Dobin et al., 2013) to align the high-quality reads to the oil palm genome (Singh et al., 2013), and HTseq to quantify expression at the gene or transcript level (Anders et al., 2015). The small RNA raw data was also submitted to the same pipeline of analysis described previously in Salgado et al. (2021); and here we generated adapter-free small RNA reads using the Cutadapt software (Martin, 2011), mapped them to the reference genome using Bowtie2 (Langmead et al., 2009). The following parameters were used to run bowtie: -a (report all alignments per read) and -V 0 (no mismatches were allowed). The oil palm genome (Singh et al., 2013) – files downloaded from NCBI (BioProject PRJNA192219; BioSample SAMN02981535) on October 2020 – was again used as reference genome.

¹ www.asd-cr.com

All adapter-free small RNA sequences (stressed and control) were concatenated into a single file for miRNA prediction. The miRNA prediction was made using mireap version 0.2² and ShortStack version 3.4,³ independently or in an association. Both programs generate clusters of sequences lined up in genomic regions. Ideally, these clusters indicate the genomic location and the miRNA precursor, mature miRNA, and miRNA* sequences. StrucVis⁴ was used in sequences classified as Y or N15 by ShortStack and/or ShortStack-mireap for structural evaluation of miRNA. At last, manual curation was made of all miRNAs classified as Y (confirmed miRNA) or N1-N15, where N15 means that the candidate has all the correct metrics, but the miRNA* is absent (Axtell and Meyers, 2018). The length of the strings, the predicted structure of the hairpin, and the annotation by homology were evaluated in miRBase using the default criteria.⁵

The prediction of miRNA-putative target genes was performed using the psRNA-Target online program, version 2,⁶ with the same parameters used in Salgado et al. (2021). The NOISeq R package (Tarazona et al., 2015) was used for the analysis of differential expression of miRNAs, having the individual counts of each sample as input. The genes that showed p -values ≥ 0.95 were designated as differentially expressed (DE).

Determination of biomass and soil water potential

Shoots and roots from three control and three drought-stressed plants, collected 14 days after setting up the treatments (DAP), were taken apart and weighed for fresh biomass determination and then dried in a forced-air oven at 65°C to constant weight to determine dry biomass. The leaf relative water content (RWC) was measured after 14 DAP in the oil palm plants; as well as the water potential (Ψ_w) of the substrate.

After harvesting the plants and homogenizing the soil, three samples were collected per pot to analyze soil water potential, using the WP4C equipment (Dew Point Potentiometer, METEER Group, Inc.). Soil samples were placed in the equipment in the accurate reading mode, as indicated by the manufacturer. The results allow the comparison between the different treatments concerning the energy state of the soil water, which refers to the soil water content. After submitting the data for normality analyses using the Shapiro–Wilk test, an statistical analysis was performed by Student's t -test at 5% probability, using GraphPad.

² <https://sourceforge.net/projects/mireap>

³ <https://github.com/MikeAxtell/ShortStack>

⁴ <https://github.com/MikeAxtell/strucVis>

⁵ <http://www.mirbase.org/search.shtml>

⁶ <https://bio.tools/psrnatarget>

Correlation analysis of differentially expressed miRNAs and mRNAs under two distinct scenarios – salinity and drought stresses

To perform correlation analysis of differentially expressed (DE) miRNAs and their respective putative target genes under two distinct scenarios, we used sets of data having the DE miRNAs and mRNAs from this present study and re-used the respective ones from Salgado et al. (2021). First, to check the data distribution, we used the Data Overview module of the Omics Fusion (Brink et al., 2016), the web platform for integrative analysis of Omics data,⁷ and then the Scatter Plot one for the correlation analysis between the sets of data—a pairwise combination of the different molecules and scenarios evaluated. The input data used was the Log₂ (FC) data of the DE miRNAs and the DE target genes obtained from the single-omics analysis.

Results

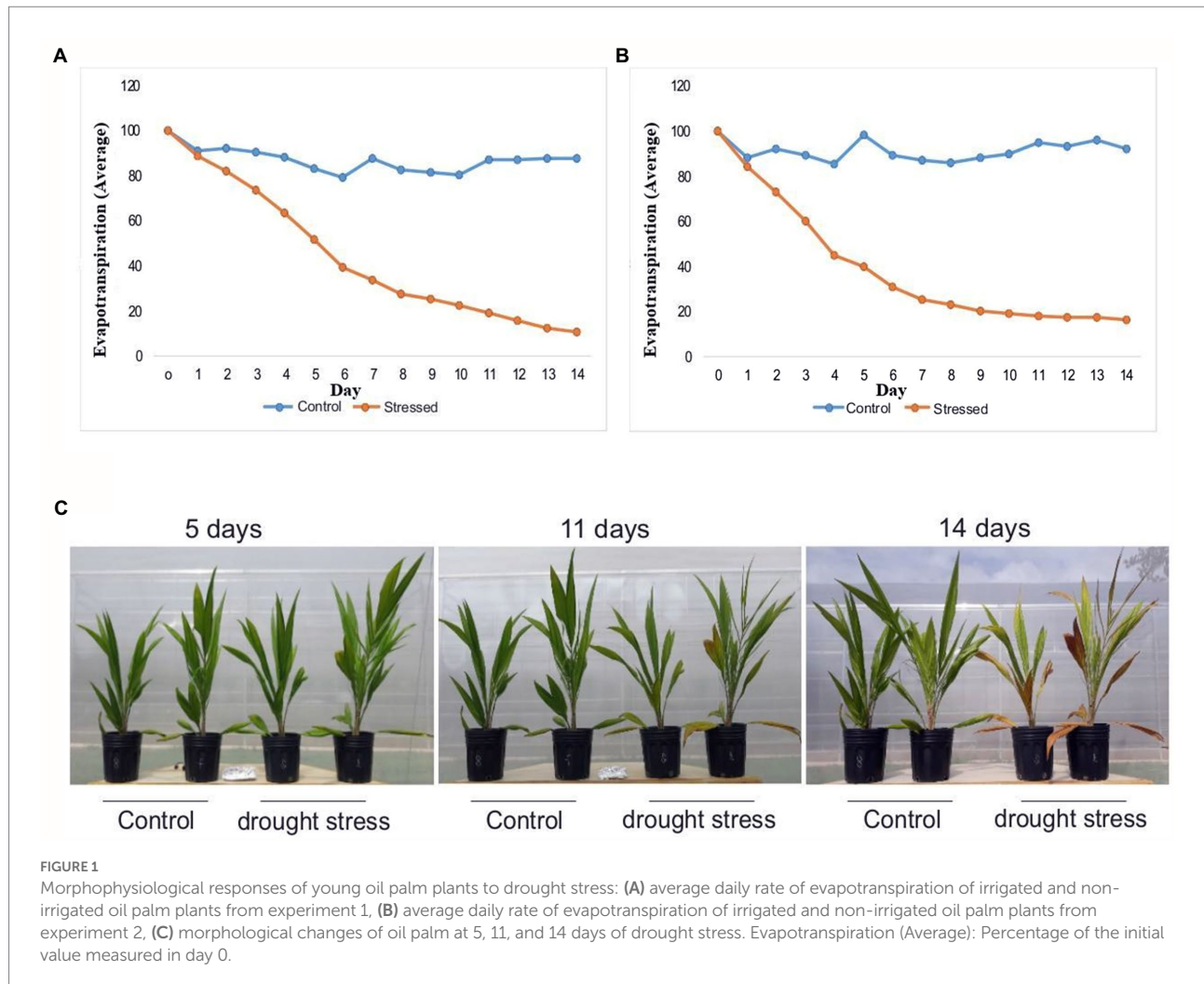
Morphophysiological responses of young oil palm plants to drought stress

The evapotranspiration rate of the control plants remained high and constant during experiments 1 and 2. Meanwhile, the water deficit caused a gradual reduction in the evapotranspiration rates in the drought-stressed plants, in both experiments (Figures 1A,B). Oil palm plants kept for 14 days under drought stress started to show morphological changes in the leaves, such as yellowing and necrosis of the edge and tip (Figure 1C). The leaf relative water content (RWC) in the oil palm plants subjected to drought stress for 14 days dropped ~50% compared to the control plants. The water potential (Ψ_w) of the substrate at the end of the experiment averaged 0.19 MPa in the control treatment and –13.60 in the drought-stressed one.

The stressed plants showed a reduction in shoots and roots fresh weight, averaging 87.19 and 61.59 g, respectively. Meanwhile, the control plants averaged 122.18 and 220.34 g, respectively. Such a significant reduction did not happen in either shoot or root dry weight, where stressed plants averaged 36.37 and 40.23 g, compared to an average of 36.85 and 55.44 g, respectively, for the control plants.

The reduction in the Ψ_w led to a reduction in most of the gas exchange parameters assessed. The net CO₂ assimilation rate (A), the stomatal conductance rates (g_s), and the transpiration rate (E), showed a significant reduction of 81.03%, 87.74%, and 86.17%, respectively, in comparison with their respective control plants (Figures 2A–C). The intracellular concentration of CO₂ (C_i) in the drought stressed plants showed a low percentage of reduction

⁷ <https://fusion.cebitec.uni-bielefeld.de>



(19.28%) when compared to the control, with no statistical difference (Figure 2D).

Identification of known and novel miRNAs and differential expression analysis of miRNAs

The raw reads generated ranged from 14.2 and 57.6 million per sample, presenting an extensive resource for discovering miRNAs (Table 1). After filtering out low-quality reads and removing adapters, the number of remaining clean reads ranged from three and 15 million. A total of 85,871,550 sequences, ranging from 20 to 24 nts in length, was then submitted to further analysis in the Rfam version 12.0 database, in order to remove non-coding RNAs – rRNA, tRNA, snRNA and snoRNA. The miRNA prediction was performed after mapping the remaining 61,666,402 small RNA sequences (Table 1) against the oil palm reference genome and concatenating them into a single file, generating 5,701 positive hits, from which 163 were Y and 5,538 were N15. The miRNA prediction was performed after mapping

the remaining 61,666,402 small RNA sequences (Table 1) against the oil palm reference genome and concatenating them into a single file, generating 5,701 positive hits, from which 163 present all necessary characteristics, including the exact miRNA-star, to be annotate as a miRNA (code Y), and 5,538 were classified as a “maybe” (N15), in accordance with miRNA analysis codes from Shortstack, requiring a manual curation.

A total of 81 miRNAs resulted from a manual curatorship evaluating the length of the strings (20–22 nts), predicting the structure of the hairpin by strucVis version 0.4,⁸ and annotation by homology in the miRBase database,⁹ being 52 conserved miRNAs already reported in other species and 29 oil palm-specific miRNAs (Table 2). It is a fact that plant miRNAs are generally 20 to 22 nt in size, with 23 and 24 being rare (Axtell and Meyers, 2018). The 24 nt sequences represent siRNAs, and none of them was pointed out as a possible miRNA by the Shortstack and mireap programs after the curation process employed in this

⁸ www.github.com/MikeAxtell/strucVis

⁹ www.mirbase.org

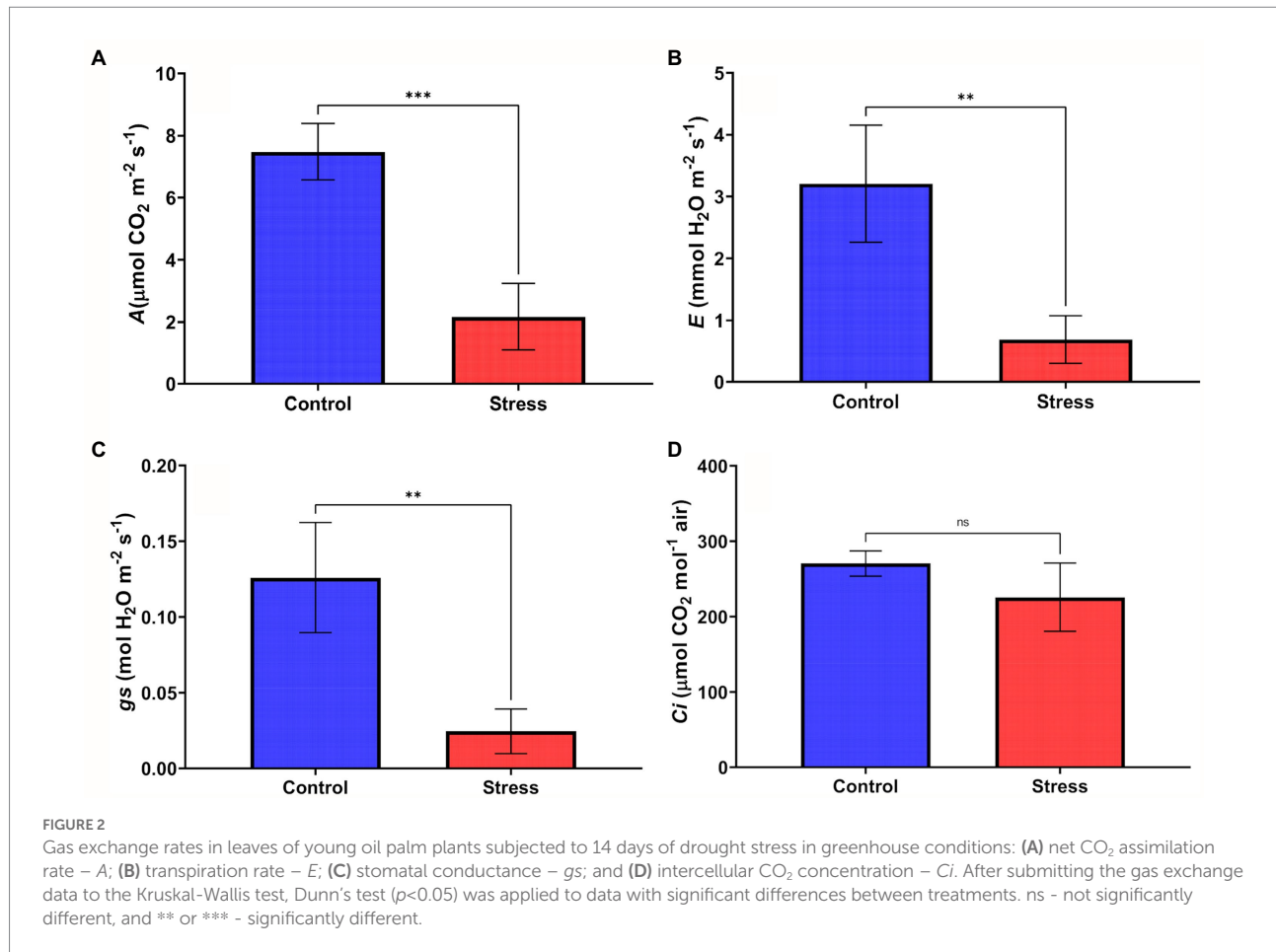


TABLE 1 Oil palm sRNA statistics and clean read length distribution.

Samples	Raw reads (%)	After Cutadapt (20–24 nt)	After Rfam (20–24 nt)
Control_R1_Lane1	17,359,260 (100%)	5,701,540 (32.84%)	4,405,869 (25.38%)
Control_R1_Lane2	19,137,482 (100%)	6,283,384 (32.83%)	4,809,227 (25.13%)
Control_R2_Lane1	19,766,560 (100%)	6,304,031 (31.89%)	5,179,368 (26.20%)
Control_R2_Lane2	21,788,523 (100%)	6,965,496 (31.97%)	5,682,583 (26.08%)
Control_R3_Lane1	25,695,279 (100%)	5,769,021 (22.45%)	3,307,121 (12.87%)
Control_R3_Lane2	28,288,139 (100%)	6,370,094 (22.52%)	3,560,220 (12.59%)
Drought_R1_Lane1	23,959,300 (100%)	6,471,468 (27.01%)	4,410,743 (18.41%)
Drought_R1_Lane2	26,514,939 (100%)	7,182,696 (27.09%)	4,824,005 (18.19%)
Drought_R2_Lane1	14,201,378 (100%)	3,063,342 (21.57%)	1,825,359 (12.85%)
Drought_R2_Lane2	15,714,905 (100%)	3,063,342 (19.49%)	1,978,270 (12.59%)
Drought_R3_Lane1	52,289,340 (100%)	13,652,218 (26.11%)	10,366,551 (19.83%)
Drought_R3_Lane2	57,646,162 (100%)	15,044,918 (26.10%)	11,317,086 (19.63%)

study, demonstrating the robustness of the prediction, annotation, and curation process used. Among the 29 new miRNAs, 27 were similar to those already reported by Salgado et al. (2021) in oil palm under salinity stress – egu-miR01sds to egu-miR27sds. The two new miRNAs identified in this study, egu-miR28ds, and egu-miR29ds, have a length of 21 nts and are located in intragenic regions of the *E. guineensis* genome (Table 2). The former is the target GTPase-activating protein GYP7 gene (LOC105043478)

and has a length of 144 nts, while the latter is in an uncharacterized protein (LOC105044755) and has 101 nts. The GTPase-activating protein GYP7 gene is 9,498 nts long, has five exons, and code for two protein isoforms in the *E. guineensis* genome. The egu-miR28ds gene is located in the third and longest exon. The uncharacterized LOC105044755, on the other hand, is 2,165 nts long and has no intron, and the egu-miR29ds gene is located pretty much in the middle of it.

TABLE 2 The two new oil palm-specific miRNAs predicted in this study, and their putative target genes predicted using psRNA-Target online program, version 2.

miRNA	miRNA size	Mireap	Shortstack	miRNA gene size
egu-miR28ds	21 nt	o	-	144 nt
Location	Gene ID*	Gene description	miRNA Aligned Fragment	
intragenic	LOC105043478	GTPase-activating protein GYP7	UAGUAGUCUCCAAAUACACAUG	
Alignment	Target Gene Aligned Fragment	Target Gene ID	Target Gene Description	
.....	5' UAUGUGAUUUGAAGACUGCUG 3'	LOC105039667	Probable protein-S-isoprenylcysteine O-methyltransferase	
.....	5' UGUGAAAUUGGAGGCUACUA 3'	LOC105050641	GBF-interacting protein 1-like	
.....	5' UGUGAAAUUGGAGGCUACUA 3'			
.....	5' UGUGAAAUUGGAGGCUACUA 3'			
.....	5' UGUGAAAUUGGAGGCUACUA 3'			
.....	5' UGUGAAAUUGGAGGCUACUA 3'			
miRNA	miRNA size	Mireap	Shortstack	miRNA gene size
egu-miR29ds	21 nt	o	o	101 nt
Location	Gene ID*	Gene description	miRNA Aligned Fragment	
intragenic	LOC105044755	Uncharacterized – protein coding	UCUCGGCGCGCCACCUCUCC	
Alignment	Target Gene Aligned Fragment	Target Gene ID	Target Gene Description	
.....	5'GGAGGAAGUCGCCGCCGAGA 3'	LOC105054028	Probable xyloglucan galactosyltransferase GT19	
.....	5'GGAAGAGGUCGCCGCCGAGC 3'	LOC109506582	Probable protein phosphatase 2C 8	
.....	5'UGAGGCGG-CGCCGCCGAGA 3'	LOC105041954	Protein FATTY ACID EXPORT 2	
.....	5'UGAGGCGG-CGCCGCCGAGA 3'	LOC105053017	Protein FATTY ACID EXPORT 7	
.....	5'GGAUGCGGUUGCCGCCGAGG 3'	LOC105041155	EF1A lysine methyltransferase 2	

To avoid the annotation of false-positive miRNAs, we followed the curation criteria suggested by Axtell and Meyers (2018), evaluating the predicted structure of the hairpin of the new miRNAs (Figure 3), both show precise excision of the initial stalk duplex of a precursor RNA, without secondary stalks or large internal loops (greater than five nts), with a processing precision above 94%.

Sixty-two out of the 81 known and novel miRNAs showed a significant (probability ≥ 0.95) different level of expression under drought stress. While miR396e, miR159b, miR529b, egu-miR19sds, and egu-miR29ds were up-regulated, the remaining 57 miRNAs had their expression level reduced, compared to the level in the control treatment (Supplementary Table 1). The ppe-miR397 miRNA appeared in different positions of the genome, with distinct differential expression levels, and, at position NC_025997.1:28868274–28868388, it was down-regulated ($p < 0.97$), while at position NC_025993.1:61023997–61024130 it did not show significant ($p < 0.77$) differential expression (Supplementary Table 1).

Prediction and differential expression analysis of miRNAs-putative target genes

The psRNA-Target online program – version 2¹⁰ – led to 357 positive hits as DE miRNA-putative target genes. Based on the LOC IDs from the oil palm reference genome (Singh et al., 2013),

there were 185 distinct putative target genes out of the 357 positive hits. All 62 DE miRNA had two or more putative target genes.

Almost 100% of the high-quality read pairs in all samples mapped to the oil palm reference genome, which had 29,567 genomic features of type “gene” retrieved from 2,781 ref. sequences in GCF_000442705.1_EG5_genomic.fna file; however, 4,115 of these features had no aligned reads detected in any of the samples (Table 3).

When comparing drought-stressed against control plants, the pairwise differential expression analysis revealed that out of the 185 distinct miRNA-putative target genes, 88 differentially expressed at False Discovery Rate (FDR) ≤ 0.05 ; being 44 positively regulated ($\text{Log}_2(\text{FC}) > 0$) and 44 negatively regulated ($\text{Log}_2(\text{FC}) < 0$). The down-regulated miRNA-putative target genes experienced a reduction in the expression level, ranging from 28% to almost 100%. On the other hand, the up-regulated miRNA-putative target genes increased from 1.28X to 166.24X (Table 4).

Integrating the expression profiles from DE miRNA-putative target genes and their respective DE miRNAs

The interaction between DE miRNAs and their putative target genes was investigated using Cytoscape – version 3.8.2,¹¹ which led to the identification of a total of 102 miRNA-mRNA

10 www.bio.tools/psrnatarget

11 <https://cytoscape.org>

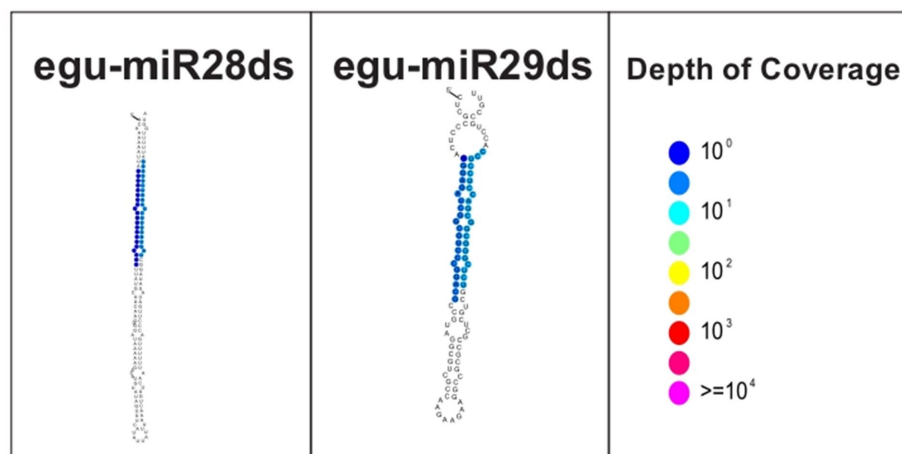


FIGURE 3
Structure of the new miRNAs identified in oil palm under water deficit.

TABLE 3 Statistics of RNA-Seq data from six samples of oil palm plants subjected to two treatments (control and drought stress); three replicates per treatment.

Sample*	Control treatment			Stressed treatment		
	R1	R2	R3	R1	R2	R3
Input high quality read pairs	28,506,037	27,758,299	27,482,363	29,085,207	29,056,879	30,875,128
Aligned reads to feature	15,462,284	23,380,536	14,294,486	17,136,348	20,031,805	25,942,349
Aligned reads to no feature	4,422,784	2,283,704	4,201,816	5,803,528	4,325,704	2,977,275
Ambiguous	230.22	401.78	225.45	199.44	228.05	315.77
Alignment not unique	8,390,751	1,692,282	8,760,612	5,945,893	4,471,325	1,639,731
Low alignment quality	0	0	0	0	0	0
Not aligned	2	2	0	0	0	0

Mapping to reference genome EG5 (BioProject PRJNA192219 and BioSample SAMN02981535) available at NCBI.

*29,567 genomic features of type “gene”, retrieved from 2,781 ref sequences in GCF_000442705.1_EG5_genomic.fna & 4,115 features (13.92%) for which no aligned reads was detected in any of the samples.

interactions, involving 62 DE miRNAs and 88 DE mRNAs. The analysis showed that a single miRNA can regulate multiple mRNAs and that a single mRNA can correlate with more than one miRNA, suggesting that the miRNA-mRNA interaction network involved in water stress is highly complex.

By integrating the expression profiles of the miRNA-putative target genes and their related miRNAs, 26 DE miRNA had just one DE putative target gene, 15 had two, eight had three, five had four, and eight had none. On the other hand, among the 88 DE putative target genes, 79 of them had just one DE miRNA, seven had two, one had three, and one had four (Table 4).

miR396e and egu-miR29ds, with a respective increment of 116% and 361%, were up-regulated due to drought stress, while their putative target genes had their expression level reduced to 25% and 71%, respectively (Table 4; Figure 4). LOC105053992 was the putative target gene that experienced the highest increase in expression, 166.24X higher than in the control plant. It experienced regulation by two miRNAs (ama-miR156 and

ata-miR156e-5p) that were down-regulated to about 50% of the level in the control plant (Figure 4).

Functional annotation of the differentially expressed target genes

The target genes underwent functional annotation using the InterProScan database. Among the 88 DE target genes selected for functional annotation, 32 had positive hits for biological processes, 50 for molecular function, and 19 for cellular components. Transmembrane transport (GO:0055085) and regulation of transcription by DNA (GO:0006355) had the highest number of biological processes present, five; followed by hormone response processes (GO: 0009725), protein phosphorylation (GO: 0006468), pigment biosynthetic process (GO: 0046148), histone lysine methylation (GO: 0034968), lipid metabolic process (GO: 0006629), oxidation-reduction process (GO: 0055114) and

TABLE 4 Profile of differential expressed miRNA and their differential expressed putative target genes from oil palm plants.

miRNA					Putative target gene				
Name	EP	Probability	FC	Log ₂ FC	ID	EP	FC	Log ₂ FC	FDR
ama-miR156	Down	1.00	0.54	-0.89	LOC105053992	Up	166.24	7.38	0.00
ata-miR156e-5p	Down	1.00	0.57	-0.81	LOC105053992	Up	166.24	7.38	0.00
bra-miR168a-5p	Down	1.00	0.25	-2.02	LOC105047586	Up	44.07	5.46	0.00
bra-miR168c-5p	Down	1.00	0.20	-2.33	LOC105047586	Up	44.07	5.46	0.00
ppe-miR397	Down	0.97	0.38	-1.40	LOC105046309	Up	30.64	4.94	0.00
egu-miR02sds	Down	1.00	0.01	-7.42	LOC105042156	Up	28.34	4.82	0.01
ppe-miR397	Down	0.97	0.38	-1.40	LOC105036954	Up	22.40	4.49	0.00
ata-miR528-5p	Down	0.99	0.24	-2.05	LOC105036904	Up	19.12	4.26	0.00
egu-miR20sds	Down	0.97	0.07	-3.91	LOC105046327	Up	14.61	3.87	0.01
ssp-miR827	Down	1.00	0.12	-3.04	LOC105043377	Up	12.69	3.67	0.00
ata-miR167d-5p	Down	1.00	0.31	-1.67	LOC105053405	Up	8.21	3.04	0.00
gma-miR482a-3p	Down	0.99	0.77	-0.38	LOC105043410	Up	7.96	2.99	0.00
egu-miR25sds	Down	0.97	0.23	-2.10	LOC105036224	Up	7.73	2.95	0.00
ata-miR166d-3p	Down	1.00	0.15	-2.78	LOC105046708	Up	6.30	2.66	0.00
atr-miR166b	Down	1.00	0.27	-1.88	LOC105046708	Up	6.30	2.66	0.00
osa-miR166i-3p	Down	1.00	0.13	-2.99	LOC105046708	Up	6.30	2.66	0.00
sly-miR166c-3p	Down	0.99	0.32	-1.63	LOC105046708	Up	6.30	2.66	0.00
ata-miR396e-5p	Down	0.97	0.49	-1.03	LOC105033560	Up	4.72	2.24	0.03
ata-miR160c-5p	Down	1.00	0.13	-2.99	LOC105044300	Up	4.66	2.22	0.00
egu-miR07sds	Down	1.00	0.23	-2.09	LOC105044919	Up	4.34	2.12	0.00
ata-miR160c-5p	Down	1.00	0.13	-2.99	LOC105038384	Up	3.49	1.80	0.00
aof-miR395a	Down	1.00	0.09	-3.45	LOC105041393	Up	3.45	1.79	0.00
atr-miR535	Down	0.99	0.57	-0.82	LOC105051200	Up	3.44	1.78	0.00
mtr-miR2673b	Down	0.99	0.03	-5.21	LOC105060921	Up	3.44	1.78	0.00
mdm-miR171b	Down	1.00	0.34	-1.57	LOC105043623	Up	3.12	1.64	0.00
osa-miR2118p	Down	1.00	0.58	-0.78	LOC105054413	Up	3.05	1.61	0.00
egu-miR04sds	Down	1.00	0.00	-7.79	LOC105040907	Up	2.86	1.51	0.00
bra-miR319-3p	Down	1.00	0.27	-1.89	LOC105032078	Up	2.71	1.44	0.00
atr-miR156c	Down	1.00	0.55	-0.87	LOC105061255	Up	2.67	1.42	0.03
bdi-miR529-5p	Down	1.00	0.24	-2.08	LOC105061255	Up	2.67	1.42	0.03
egu-miR07sds	Down	1.00	0.23	-2.09	LOC105061572	Up	2.59	1.37	0.00
osa-miR2118p	Down	1.00	0.58	-0.78	LOC105054674	Up	2.50	1.32	0.00
egu-miR18sds	Down	0.97	0.32	-1.63	LOC105055689	Up	2.45	1.29	0.00
egu-miR15sds	Down	0.99	0.32	-1.64	LOC105044088	Up	2.41	1.27	0.00
egu-miR05sds	Down	1.00	0.12	-3.09	LOC105045735	Up	2.40	1.26	0.00
egu-miR10sds	Down	1.00	0.15	-2.70	LOC105043130	Up	1.78	0.83	0.00
ata-miR528-5p	Down	0.99	0.24	-2.05	LOC105055547	Up	1.77	0.82	0.00
aof-miR395a	Down	1.00	0.09	-3.45	LOC105038678	Up	1.67	0.74	0.00
aof-miR391	Down	0.99	0.09	-3.53	LOC105045520	Up	1.63	0.71	0.00
egu-miR11sds	Down	0.99	0.08	-3.62	LOC105050858	Up	1.63	0.70	0.00
egu-miR10sds	Down	1.00	0.15	-2.70	LOC105034850	Up	1.62	0.69	0.00
ssp-miR827	Down	1.00	0.12	-3.04	LOC105034273	Up	1.60	0.68	0.00
egu-miR02sds	Down	1.00	0.01	-7.42	LOC105047587	Up	1.60	0.68	0.01
ata-miR169i-3p	Down	1.00	0.07	-3.92	LOC105043671	Up	1.60	0.68	0.00
ata-miR528-5p	Down	0.99	0.24	-2.05	LOC105034839	Up	1.52	0.61	0.00
ata-miR169i-3p	Down	1.00	0.07	-3.92	LOC105060868	Up	1.47	0.55	0.00
egu-miR08sds	Down	1.00	0.11	-3.22	LOC105042656	Up	1.45	0.53	0.00
bra-miR168a-5p	Down	1.00	0.25	-2.02	LOC105051551	Up	1.39	0.48	0.01
bra-miR168a-5p	Down	1.00	0.25	-2.02	LOC105042722	Up	1.28	0.36	0.04

(Continued)

TABLE 4 (Continued)

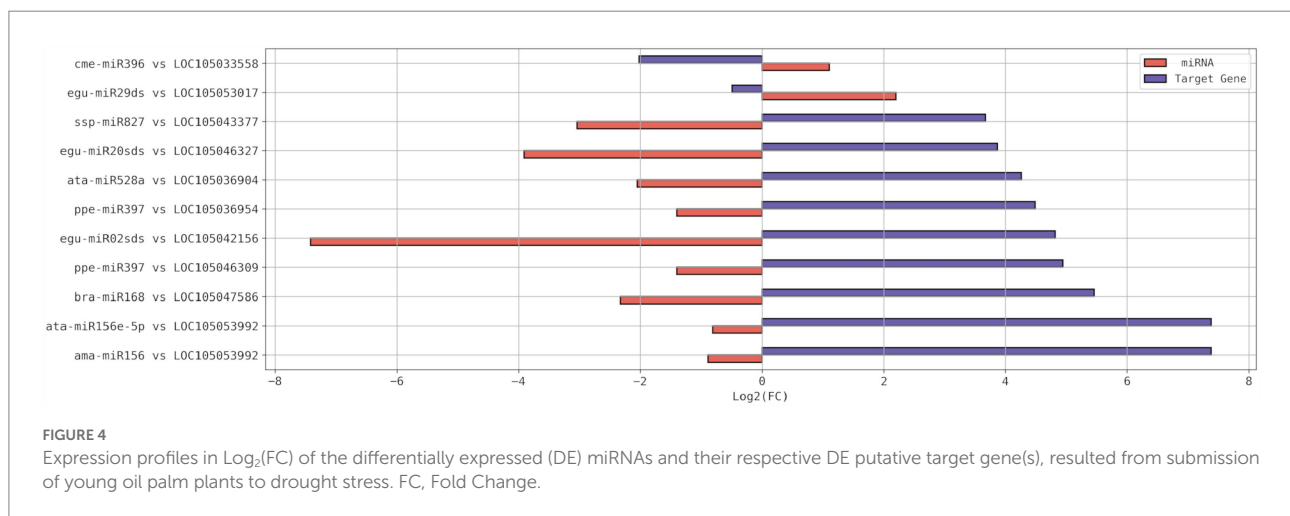
miRNA		Putative target gene							
Name	EP	Probability	FC	Log ₂ FC	ID	EP	FC	Log ₂ FC	FDR
ssp-miR827	Down	1.00	0.12	-3.04	LOC105054157	Down	0.00	-7.75	0.00
ata-miR169i-3p	Down	1.00	0.07	-3.92	LOC105051537	Down	0.03	-5.26	0.00
ata-miR399a-3p	Down	1.00	0.01	-6.21	LOC105035561	Down	0.03	-5.13	0.00
ata-miR395c-3p	Down	1.00	0.08	-3.64	LOC105048606	Down	0.04	-4.60	0.00
egu-miR20sds	Down	0.97	0.07	-3.91	LOC105032247	Down	0.06	-4.18	0.00
egu-miR08sds	Down	1.00	0.11	-3.22	LOC105048722	Down	0.14	-2.82	0.00
atr-miR159	Down	1.00	0.23	-2.13	LOC105043705	Down	0.16	-2.65	0.00
egu-miR05sds	Down	1.00	0.12	-3.09	LOC105041381	Down	0.16	-2.61	0.00
egu-miR03sds	Down	0.99	0.48	-1.05	LOC105059001	Down	0.17	-2.58	0.00
aof-miR391	Down	0.99	0.09	-3.53	LOC105039220	Down	0.17	-2.57	0.00
mes-miR399f	Down	1.00	0.01	-6.62	LOC105052027	Down	0.20	-2.29	0.00
egu-miR09sds	Down	1.00	0.21	-2.22	LOC105041147	Down	0.22	-2.21	0.00
ata-miR396e-5p	Down	0.97	0.49	-1.03	LOC105033558	Down	0.25	-2.02	0.00
ata-miR528-5p	Down	0.99	0.24	-2.05	LOC105048962	Down	0.29	-1.77	0.00
ata-miR169i-3p	Down	1.00	0.07	-3.92	LOC105047549	Down	0.31	-1.71	0.03
ata-miR390-5p	Down	0.98	0.22	-2.20	LOC105053593	Down	0.31	-1.70	0.00
bdi-miR169c-3p	Down	1.00	0.08	-3.67	LOC105046060	Down	0.33	-1.61	0.03
gma-miR482a-3p	Down	0.99	0.77	-0.38	LOC105058639	Down	0.33	-1.58	0.00
egu-miR01sds	Down	1.00	0.16	-2.60	LOC105055591	Down	0.34	-1.56	0.00
egu-miR04sds	Down	1.00	0.00	-7.79	LOC105052116	Down	0.36	-1.47	0.00
ata-miR167d-5p	Down	1.00	0.31	-1.67	LOC105049211	Down	0.38	-1.41	0.00
aof-miR395a	Down	1.00	0.09	-3.45	LOC105042288	Down	0.38	-1.41	0.00
vvi-miR828a	Down	0.96	0.58	-0.78	LOC105051514	Down	0.41	-1.28	0.00
ata-miR396e-5p	Down	0.97	0.49	-1.03	LOC105052992	Down	0.44	-1.20	0.00
ata-miR396e-5p	Down	0.97	0.49	-1.03	LOC105042167	Down	0.48	-1.05	0.00
egu-miR11sds	Down	0.99	0.08	-3.62	LOC105056373	Down	0.49	-1.02	0.00
egu-miR26sds	Down	0.97	0.13	-2.91	LOC105056373	Down	0.49	-1.02	0.00
mdm-miR171b	Down	1.00	0.34	-1.57	LOC105059511	Down	0.49	-1.02	0.00
egu-miR18sds	Down	0.97	0.32	-1.63	LOC105031985	Down	0.50	-1.01	0.00
bdi-miR530b	Down	0.99	0.21	-2.23	LOC105055997	Down	0.50	-1.00	0.00
mdm-miR171b	Down	1.00	0.34	-1.57	LOC105054013	Down	0.53	-0.92	0.00
mdm-miR171b	Down	1.00	0.34	-1.57	LOC105054987	Down	0.58	-0.79	0.00
egu-miR15sds	Down	0.99	0.32	-1.64	LOC105050166	Down	0.58	-0.78	0.01
vvi-miR828a	Down	0.96	0.58	-0.78	LOC105043777	Down	0.59	-0.77	0.00
vvi-miR828a	Down	0.96	0.58	-0.78	LOC105039801	Down	0.61	-0.70	0.00
egu-miR11sds	Down	0.99	0.08	-3.62	LOC105049000	Down	0.62	-0.69	0.01
egu-miR03sds	Down	0.99	0.48	-1.05	LOC105048718	Down	0.63	-0.66	0.00
egu-miR08sds	Down	1.00	0.11	-3.22	LOC105049925	Down	0.63	-0.66	0.00
egu-miR03sds	Down	0.99	0.48	-1.05	LOC105060476	Down	0.67	-0.58	0.00
egu-miR07sds	Down	1.00	0.23	-2.09	LOC105058953	Down	0.68	-0.56	0.02
egu-miR22sds	Down	0.99	0.06	-4.18	LOC105060538	Down	0.68	-0.56	0.02
ata-miR399a-3p	Down	1.00	0.01	-6.21	LOC105047412	Down	0.68	-0.56	0.00
mes-miR399f	Down	1.00	0.01	-6.62	LOC105047412	Down	0.68	-0.56	0.00
aof-miR395a	Down	1.00	0.09	-3.45	LOC105041383	Down	0.71	-0.50	0.00
osa-miR2118p	Down	1.00	0.58	-0.78	LOC105044139	Down	0.72	-0.48	0.02
cme-miR396e	Up	0.97	2.16	1.11	LOC105033558	Down	0.25	-2.02	0.00
egu-miR29ds	Up	0.97	4.61	2.20	LOC105053017	Down	0.71	-0.49	0.01
cme-miR396e	Up	0.97	2.16	1.11	LOC105033560	Up	4.72	2.24	0.03

(Continued)

TABLE 4 (Continued)

miRNA					Putative target gene				
Name	EP	Probability	FC	Log ₂ FC	ID	EP	FC	Log ₂ FC	FDR
egu-miR19sds	Up	0.95	1.45	0.54	LOC105049219	Up	3.62	1.86	0.00
cpa-miR159b	Up	0.97	2.32	1.21	LOC105032078	Up	2.71	1.44	0.00
osa-miR529b	Up	0.98	3.28	1.71	LOC105061255	Up	2.67	1.42	0.03

False discovery rate (FDR), Identity in the reference genome EG5 at NCBI (ID), and fold change (FC). EP, Expression profile; FC, Fold Change (Drought/Control).



histone methylation (GO: 0016571), with two each (Supplementary Table 2).

For molecular functions, the GO terms that appeared the most were protein binding (GO: 0005515), with nine occurrences, followed by DNA binding (GO: 0003677), with eight, and ATP binding (GO: 0005524), with seven. The cellular component most frequently present was the nucleus (GO: 0005634), with seven occurrences, followed by the membrane (GO: 0016020), with six, and the integral membrane component (GO: 0016021), with four (Supplementary Table 2).

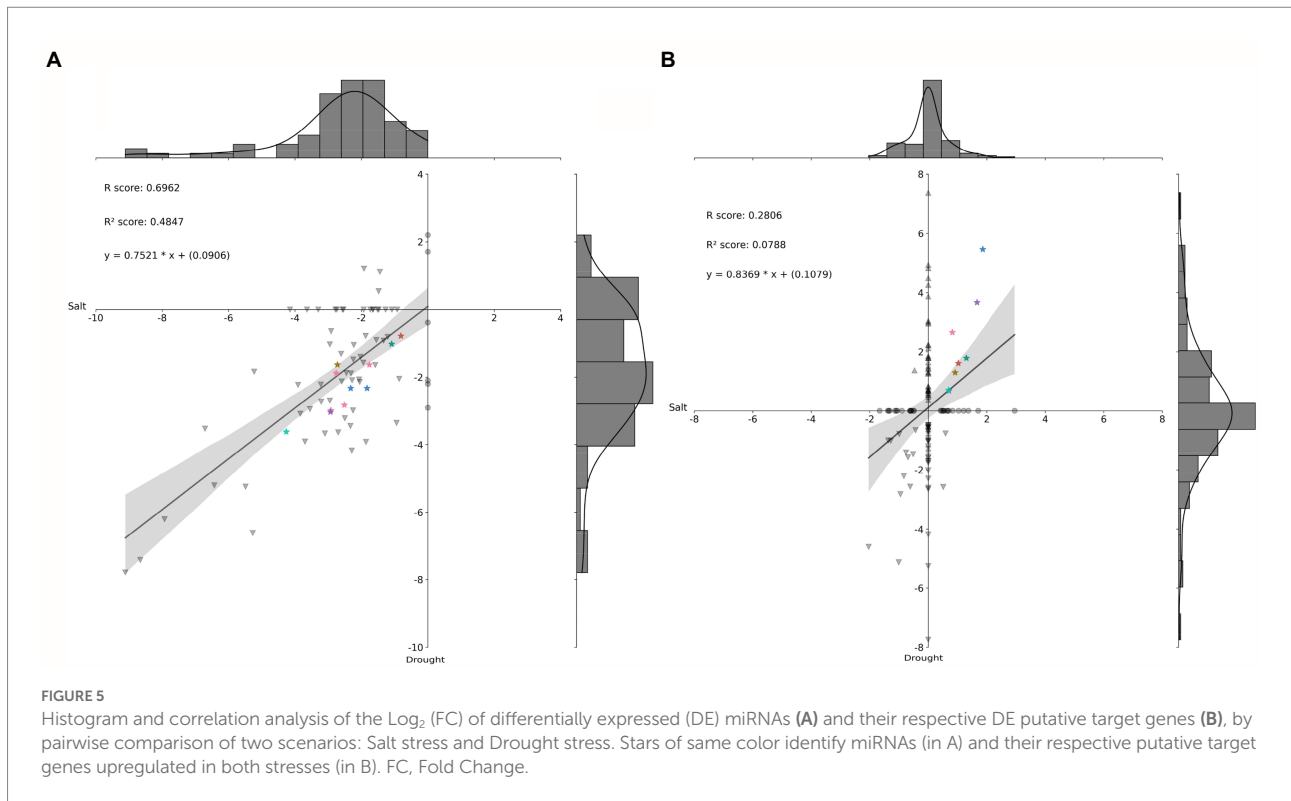
When analyzing the most expressed domains, TF_GRAS (IPR005202) came first with 4, followed by SPX domain (IPR004331) with three, and SET_dom (IPR001214), Tyrosinase_Cu-bd (IPR002227), Protein kinase domain (IPR000719), SANT/Myb (IPR001005), and SBP_dom (IPR004333) with two hits each. MFS family, with three members, was the largest one, followed by the ABC, Polyphenol_oxidase, and Hist-Lys_N-MeTrfase_plant families with two members each (Supplementary Table 2).

To further characterize the putative target genes, the LOC numbers were used to identify the ID of the protein coded by them. Nine LOC numbers were from genes coding for ncRNAs, and the remaining 79 led to the identification of 138 proteins (distinct XP id). These 138 proteins were submitted to analysis in the GhostKOALA platform (Kanehisa et al., 2016), and in the EggNOG v5.0 platform (Huerta-Cepas et al., 2019; Cantalapiedra

et al., 2021). The results of the hierarchical, functional and phylogenetic annotation using the EggNOG platform, having the Liliopsida class as taxonomic scope, is presented in Supplementary Table 3; only 132 out of the 138 proteins were annotated. Only 44 proteins were annotated in the GhostKOALA platform, being 11 transcription factors from three families – MYB, HOX, and NF-Y.

Correlation analyses of DE miRNA-putative target genes and their respective DE miRNAs

First, 72 miRNAs differentially expressed in young oil palm plants under salinity stress (Salgado et al., 2021) were submitted to correlation analysis against 62 from the present study (Figure 5A). Then, 51 DE miRNA-putative target genes from Salgado et al. (2021) underwent correlation analysis against the 88 from the present study (Figure 5B). These results revealed eight miRNA-putative target genes upregulated in both scenarios, salinity and drought stress (Figure 5B), that also had the miRNAs targeting them down-regulating in those scenarios (Figure 5A). Among them, there were three genes expressing lncRNAs, two coding for proteins from the Major facilitator superfamily, one for a putative histone-modifying enzyme harboring a demethylase



activity, one for a protein from the class III homeodomain-leucine zipper family, and one for a protein from the Macrophage migration inhibitory factor (MIF) family.

Discussion

Palms are considered the third most important economic group of plants, second only to grasses and legumes (Meerow et al., 2012). miRNAs have been described as master regulators of gene expression and involved in controlling growth and development and plant responses to different stresses (Liu et al., 2008; Ding et al., 2009; Djami-Tchatchou et al., 2017). Still, little information on palm miRNA is available (Md Nasaruddin et al., 2007; Low et al., 2014; da Silva et al., 2016; Ho et al., 2017; Zheng et al., 2019; Salgado et al., 2021), and none of them studied the miRNAs involved in the response of oil palm plants to drought stress. Thus, this is the first report of prospecting oil palm miRNAs and analyzing their expression profile together with their target genes under water deprivation.

The reduction of g_s and Ψ_w seen in the present study, inducing an abrupt stomatal closure, is a shred of evidence that oil palm has a high sensitivity to drought. It suggests that mechanisms for water control mediate the decrease in g_s ; in other words, that water deprivation is sensed by the roots, triggering the production of abscisic acid (ABA), which results in stomatal closure mediated by a transduction cascade (Chaves et al., 2009; Cutler et al., 2010; Silva et al., 2016). The effects of A reduction during water deficit (Figure 2A) may be related to the increase in diffusive (stomatal

and mesophilic restrictions) and biochemical limitations (Chaves et al., 2009; Flexas et al., 2012).

This study identified 81 miRNAs, where 52 miRNAs are deeply conserved among various plant species, such as *A. thaliana* (Liu et al., 2008), *O. sativa* (Zhou et al., 2010), *Populus trichocarpa* (Lu et al., 2008), and *M. truncatula* (Wang et al., 2011). Some of them, miR156, miR160, miR166, miR167, miR168, miR172, miR396, miR528, and miR535, have already been found in oil palm by Ho et al. (2017) in floral meristems, while Fang et al. (2013) identified miR156, miR395, and miR528 in the mesocarp of oil palm fruits. Also, Md Nasaruddin et al. (2007) identified miR156, miR159, and miR160 in oil palm's apical meristem and immature and mature flowers. The other 29 miRNAs are specific for oil palm (Table 3), from which 27 have been already reported in our previous study (Salgado et al., 2021) – regarding oil palm responses to salinity stress, and two (egu-miR28ds and egu-miR29ds) reported for the first time.

Sixty-two out of the 81 miRNAs showed significant differential expression. While five of them up-regulated under drought stress, the remaining 57 were down-regulated (Table 4). That is a behavior similar to the one seen usually in genes encoding proteins – meaning that a miRNA expression is also up- or down-regulated in response to stress (Fang et al., 2013), as also shown for *A. thaliana* (Liu et al., 2008), *O. sativa* (Zhou et al., 2010), *P. trichocarpa* (Lu et al., 2008), *M. truncatula* (Wang et al., 2011), among others.

A series of transcription factor (TFs) putative genes were also targeted by miRNAs in such plants when submitted to drought stress, as already seen in young oil palm plants under salinity

stress (Salgado et al., 2021). TFs, such as auxin response factors (ARFs), squamosa promoter binding proteins, ethylene response transcription factors, MYB transcription factor, homeobox-leucine protein zipper HOX, transcription factor GAMYB, and nuclear transcription factor Y, which are responsible for regulating plant growth and development (Wu and Poethig, 2006; Gandikota et al., 2007), were predicted as putative miRNA-target genes in oil palm under water deficit in this study.

Most of these miRNAs targeting TFs were down-regulated (miR156, miR160c-5p, miR166d-3p, miR169i-3p, miR156c, miR166b, miR529-5p, miR319-3p, egu-miR02sds, egu-miR07sds, egu-miR11sds, egu-miR26sds, miR171b, miR166i-3p, miR166c-3p), indicating that the decrease in these miRNAs will increase the expression of some corresponding transcription factors, thus promoting the activation of a set of encoding genes to play defensive roles against abiotic stresses. This behavior is present in other species under water deficit (Liu et al., 2008; Zhou et al., 2010).

The plant response to drought stress is very similar to the initial plant response to salinity stress, and many common occurrences between these two primary stresses are also expected (Uddin et al., 2016). Salgado et al. (2021) observed that miRNAs miR166, miR169, miR319, miR396, and miR529 shows reduced expression profiles in young oil palm plants subjected to salt stress, a similar behavior to what happened in oil palm plants under drought stress. It is proved, through functional analysis, that these miRNAs regulate the levels of transcription of TFs, thus affecting the levels of TF proteins.

miR529 in the leaves of oil palm plants under salinity (Salgado et al., 2021) and drought stress showed a reduction of 80% and 76% in expression levels, respectively. Both showed up-regulation of their putative target gene – SBP (squamosa promoter-binding-like protein) – by ~70%. Squamosa promoter binding proteins are putative transcription factors with a plant-specific SBP domain consisting of 76 amino acids in length responsible for regulating various biological processes, including drought and saline stress. According to studies carried out by Hou et al. (2018), the overexpression of the homologous SBP16 gene from grapes (*Vitis vinifera*) in *A. thaliana* promoted an increase in tolerance to drought and salinity stresses during seed germination, as well as in seedlings and mature plants, regulating the signaling cascades of SOS and ROS.

egu-miR02sds showed a 99% reduction in its expression level in plants under drought stress, promoting a 60% increase in the expression of its putative target gene NF-YB (LOC105047587 – nuclear transcription factor Y subunit B-4-like), a similar behavior shown when under salt stress (Salgado et al., 2021). The Nuclear factor Y (NF-Y) is a ubiquitous transcription factor with high affinity and sequence specificity for the CCAAT box, a cis-element present in about 25% of eukaryotic gene promoters. NF-Y is a heterotrimeric complex composed of three distinct subunits (NF-YA, NF-YB, and NF-YC; Mantovani, 1999). Studies shows that NF-Y is responsible for activating a transcriptional cascade critical for drought resistance, where its overexpression improves

resistance and promotes drought tolerance in arabidopsis and maize (Nelson et al., 2007; Liu et al., 2008).

According to Li et al. (2008), overexpression of NF-YA5 in arabidopsis reduced water loss in leaves and resulted in a resistance to water stress higher than the wild type. Nelson et al. (2007) showed that NF-YB overexpression promoted drought tolerance in maize, based on the responses of several stress-related parameters, including chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis. Thus, NF-Y is crucial for expressing a series of genes responsive to drought stress, and its induction takes place at both transcriptional and post-transcriptional levels (Li et al., 2008; Liu et al., 2008).

The miRNAs miR168, miR395, miR396, miR397 were also down-regulated and are directly involved in the stress response process or stress tolerance (Sunkar et al., 2005; Gray-Mitsumune and Matton, 2006). miR168 regulates the *Argonaute 1* (AGO1) gene in *A. thaliana* and *P. trichocarpa* (Lu et al., 2005). AGO1 proteins can be directly associated with siRNAs / miRNAs before and after recognizing their mRNA targets and are required for normal plant development (Vaucheret et al., 2004). In the present study, miR168 expression level experienced a 75% reduction in drought-stressed plants, while its putative target gene (LOC105042722 – protein argonaute 1A-like) up-regulated 28%.

The present study led us to identify two new oil palm-specific miRNAs and enabled a comparative analysis of expression levels with miRNAs in oil palm reported under salt stress (Salgado et al., 2021). Comparative studies of miRNAs can help to increase our understanding, at a regulatory level, of the events that give rise to new species or the emergence of specific characteristics. The correlation analysis done in this present study showed that lncRNAs might play some role in the response of young oil palm plants to both drought and salinity stresses. Long non-coding RNAs (lncRNAs) are >200 nucleotides-long RNAs that are not translated into functional proteins but have cellular functions of a structural and/or regulatory nature (Statello et al., 2021). Accordingly to Jha and colleagues, lncRNAs play an essential role in plant adaptation to various abiotic stresses, such as drought, heat, cold, heavy metal toxicity, and nutrient deficiency (Jha et al., 2020).

The Major Facilitator Superfamily (MFS) is the largest group of secondary active membrane transporters that shows a much larger number of genes in plant genomes than in bacteria, yeast, or animals (Niño-González et al., 2019). Again, the correlation analysis done in this present study showed that an MFS gene plays a role in the response of young oil palm plants to both abiotic stresses under consideration. Genes from the MFS are involved in plant response to abiotic stress in arabidopsis, mediating drought and salt tolerance (Remy et al., 2013; Wang, D. et al., 2020).

Finally, regarding the remaining three proteins shown to be potentially playing a role in the response of young oil palm plant to drought and salinity stresses – a putative histone-modifying enzyme harboring a demethylase activity, a protein from the class III homeodomain-leucine zipper family, and a

protein from the MIF family, reports are there linking them to stress memory (Bhadouriya et al., 2021), regulation of defense response to abiotic and biotic stresses (Sharif et al., 2021), and modulation of ROS signaling (Zhao et al., 2021).

Many studies are available where the main objective is to compare the effects of those or more two types of stresses on plant growth and development (Chaves et al., 2009; de Oliveira et al., 2013; Uddin et al., 2016; Ma et al., 2020). This present study allowed us to visualize some of the similarities and dissimilarities – regarding expression analysis of miRNAs and their putative target genes – young oil palm plants have with the response to two highly important environmental stresses, drought and salinity. As far as we know, this is the first time such type of study is done in oil palm.

The present study and our previous one (Salgado et al., 2021) used plants with the same genetic background (clones from the same plant), the same age (young oil palm plants), almost similar duration of stress (12 and 14 days, respectively for salinity and drought stress), same omics platform (transcriptome of mRNAs and smallRNAs), and the same group of analytical tools. All these commonalities between these two studies allowed us analyze the similarities and dissimilarities regarding expression analysis of miRNAs and their putative target genes, what it a valuable set of information to help us in the search for genes that can be used to promote future attempts to horizontally transfer tolerance at once to both stresses; not only to oil palm, but also other plant species (Patel et al., 2019; Chaudhary et al., 2021).

Conclusion

This study characterized the miRNA population and their miRNA-target genes present in the leaves of young oil palm plants exposed to drought stress, besides it performed correlation analysis of the miRNAs and their target genes differentially expressed under drought and salinity stresses (from Salgado et al., 2021). Together, those activities resulted in:

- (a) The identification of two new miRNAs that received the names egu-miR28ds and egu-miR29ds, where egu is the abbreviation of *Elaeis guineensis* and ds stands for drought stress;
- (b) The prediction of 185 distinct genes as the targets to the 81 miRNAs in the genome of oil palm and a total of 102 miRNA-mRNA interactions involving 62 DE miRNAs and 88 DE mRNAs;
- (c) Among the 88 DE target genes selected for functional annotation, 32 had positive hits for biological processes, 50 for molecular function, and 19 for cellular components; and.
- (d) Eight miRNA-putative target genes – upregulated under salinity as well as drought stress – that code for lncRNAs, proteins from the Major facilitator superfamily, a putative histone-modifying enzyme harboring a demethylase activity, a protein from the class III homeodomain-leucine zipper family, and a protein from the Macrophage migration inhibitory factor (MIF) family.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/>, The smallRNA raw sequence data used in this study have been uploaded in the SRA database of the NCBI under *Elaeis guineensis* microRNA_Drought and Salinity Stresses – BioProject PRJNA646488 (SUB7775347), BioSample SAMN12799239 (SUB6325749), SRA submission SUB7897143 (accessions from SRR12424937 to SRR12424945). All RNA-seq fastq files used in this study have been uploaded in the SRA database of the NCBI under *Elaeis guineensis* Transcriptome_Drought and Salinity Stresses – BioProject PRJNA573093 (SUB6324604), BioSample SAMN12799239 (SUB6325749), SRA submission SUB6335775 (accessions from SRR10219424 to SRR10219441). The data-sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

CS and MS designed the study. FS, TS, LV, VS, and AL performed the experiments and generated the data. FS, PG, MC, RT, CS, and MS analyzed the data. FS, PG, CS, and MS wrote first draft of the manuscript, which was extensively edited and approved the submitted version by all authors. MS was responsible for the funding acquisition, project administration, and group supervision. All authors contributed to the article and approved the submitted version.

Funding

The grant (01.13.0315.00—DendePalm Project) for this study was awarded by the Brazilian Ministry of Science, Technology, and Innovation (MCTI) via the Brazilian Research and Innovation Agency (FINEP). The authors confirm that the funder had no influence over the study design, the content of the article, or the selection of this journal.

Acknowledgments

The authors acknowledge funding to FS, TS, LV, and VS by the Coordination for the Improvement of Higher Education Personnel (CAPES), via the Graduate Program in Plant Biotechnology at the Federal University of Lavras (UFLA).

Conflict of interest

AL, MC, RT, CS, PG, and MS were employed by The Brazilian Agricultural Research Corporation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.970113/full#supplementary-material>

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